EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	2013	Phytase and mutant?	US-PGPUB; USPAT; DERWENT	OR	ON	2006/06/23 12:33
L2	14732	mutant and "228"	US-PGPUB; USPAT; DERWENT	OR	ON	2006/06/23 12:34
L3	166	l1 and l2	US-PGPUB; USPAT; DERWENT	OR	ON	2006/06/23 12:34
L4	2	l1 and l2 and E228	US-PGPUB; USPAT; DERWENT	OR	ON	2006/06/23 12:36
L5	1	l1 and l2 and E228q	US-PGPUB; USPAT; DERWENT	OR	ON	2006/06/23 12:35
L6	1	l1 and l2 and double adj substitution	US-PGPUB; USPAT; DERWENT	OR	ON	2006/06/23 12:37
L7	1	l1 and l2 and mutant adj phytase	US-PGPUB; USPAT; DERWENT	OR	ON	2006/06/23 12:37
L8	10	l1 and l2 and mutant adj10 phytase	US-PGPUB; USPAT; DERWENT	OR	ON	2006/06/23 12:40
L9	76	l1 and l2 and aspergillus adj niger adj phytase	US-PGPUB; USPAT; DERWENT	OR	ON	2006/06/23 12:45
L10	1528	aspergillus adj niger adj phytase and mutant	US-PGPUB; USPAT; DERWENT	OR	ON	2006/06/23 12:42
L11	0	modifyied adj10 aspergillus adj niger adj phytase	US-PGPUB; USPAT; DERWENT	OR	ON	2006/06/23 12:43
L12	1	e228 and aspergillus adj niger adj phytase	US-PGPUB; USPAT; DERWENT	OR	ON	2006/06/23 12:45

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=> logoff
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:n

=> s phytase and mutant
            752 PHYTASE
            127 PHYTASES
            760 PHYTASE
                  (PHYTASE OR PHYTASES)
         169375 MUTANT
          98956 MUTANTS
         225610 MUTANT
                  (MUTANT OR MUTANTS)
L7           25 PHYTASE AND MUTANT

=> d ibib abs l7 1-25

L7   ANSWER 1 OF 25      MEDLINE on STN
ACCESSION NUMBER:    2006329899      IN-PROCESS
DOCUMENT NUMBER:     PubMed ID: 16751556
TITLE:               Shifting the pH profile of Aspergillus niger PhyA
                     phytase to match the stomach pH enhances its
                     effectiveness as an animal feed additive.
AUTHOR:              Kim Taewan; Mullaney Edward J; Porres Jesus M; Roneker Karl
                     R; Crowe Sarah; Rice Sarah; Ko Taegu; Ullah Abul H J; Daly
                     Catherine B; Welch Ross; Lei Xin Gen
CORPORATE SOURCE:    Department of Animal Science, Cornell University, Ithaca,
                     NY 14853, USA.
SOURCE:              Applied and environmental microbiology, (2006 Jun) Vol. 72,
                     No. 6, pp. 4397-403.
                     Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY:        United States
DOCUMENT TYPE:       Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE:          Entered STN: 6 Jun 2006
                     Last Updated on STN: 22 Jun 2006
AB   Environmental pollution by phosphorus from animal waste is a major problem
     in agriculture because simple-stomached animals, such as swine, poultry,
     and fish, cannot digest phosphorus (as phytate) present in plant feeds.
     To alleviate this problem, a phytase from Aspergillus niger PhyA
     is widely used as a feed additive to hydrolyze phytate-phosphorus.
     However, it has the lowest relative activity at the pH of the stomach
     (3.5), where the hydrolysis occurs.  Our objective was to shift the pH
     optima of PhyA to match the stomach condition by substituting amino acids
     in the substrate-binding site with different charges and polarities.
     Based on the crystal structure of PhyA, we prepared 21 single or multiple
     mutants at Q50, K91, K94, E228, D262, K300, and K301 and expressed
     them in Pichia pastoris yeast.  The wild-type (WT) PhyA showed the unique
     bihump, two-pH-optima profile, whereas 17 mutants lost one pH
     optimum or shifted the pH optimum from pH 5.5 to the more acidic side.
     The mutant E228K exhibited the best overall changes, with a
     shift of pH optimum to 3.8 and 266% greater (P < 0.05) hydrolysis of soy
     phytate at pH 3.5 than the WT enzyme.  The improved efficacy of the enzyme
     was confirmed in an animal feed trial and was characterized by biochemical
     analysis of the purified mutant enzymes.  In conclusion, it is
     feasible to improve the function of PhyA phytase under stomach
     pH conditions by rational protein engineering.

L7   ANSWER 2 OF 25      MEDLINE on STN
ACCESSION NUMBER:    2006138363      MEDLINE
DOCUMENT NUMBER:     PubMed ID: 16280324
TITLE:               Conserved role of the linker alpha-helix of the bacterial
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disulfide isomerase DsbC in the avoidance of misoxidation by DsbB.

AUTHOR: Segatori Laura; Murphy Lori; Arredondo Silvia; Kadokura Hiroshi; Gilbert Hiram; Beckwith Jon; Georgiou George

CORPORATE SOURCE: Department of Chemical Engineering, Institute for Cell and Molecular Biology, University of Texas, Austin, Texas 78712-1095, USA.

CONTRACT NUMBER: 41883 (NIGMS)
GM55090

SOURCE: The Journal of biological chemistry, (2006 Feb 24) Vol. 281, No. 8, pp. 4911-9. Electronic Publication: 2005-11-09.
Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200605
ENTRY DATE: Entered STN: 11 Mar 2006
Last Updated on STN: 5 May 2006
Entered Medline: 4 May 2006

AB In the bacterial periplasm the co-existence of a catalyst of disulfide bond formation (DsbA) that is maintained in an oxidized state and of a reduced enzyme that catalyzes the rearrangement of mispaired cysteine residues (DsbC) is important for the folding of proteins containing multiple disulfide bonds. The kinetic partitioning of the DsbA/DsbB and DsbC/DsbD pathways partly depends on the ability of DsbB to oxidize DsbA at rates >1000 times greater than DsbC. We show that the resistance of DsbC to oxidation by DsbB is abolished by deletions of one or more amino acids within the alpha-helix that connects the N-terminal dimerization domain with the C-terminal thioredoxin domain. As a result, **mutant** DsbC carrying alpha-helix deletions could catalyze disulfide bond formation and complemented the phenotypes of dsbA cells. Examination of DsbC homologues from Haemophilus influenzae, Pseudomonas aeruginosa, Erwinia chrysanthemi, Yersinia pseudotuberculosis, Vibrio cholerae (30-70% sequence identity with the Escherichia coli enzyme) revealed that the mechanism responsible for avoiding oxidation by DsbB is a general property of DsbC family enzymes. In addition we found that deletions in the linker region reduced, but did not abolish, the ability of DsbC to assist the formation of active vtPA and **phytase** in vivo, in a DsbD-dependent manner, revealing that interactions between DsbD and DsbC are also conserved.

L7 ANSWER 3 OF 25 MEDLINE on STN

ACCESSION NUMBER: 2006082303 IN-PROCESS

DOCUMENT NUMBER: PubMed ID: 16468358

TITLE: Improving thermostability of Aspergillus niger **phytase** by elongation mutation.

AUTHOR: Chen Hui; Wang Hong-Ning; Yang Wan-Shen; Zhao Hai-Xia; Wu Qi; Shan Zhi

CORPORATE SOURCE: College of Life & Science, Sichuan Agriculture University, Ya' an, Sichuan 625014.

SOURCE: Sheng wu gong cheng xue bao = Chinese journal of biotechnology, (2005 Nov) Vol. 21, No. 6, pp. 983-7.
Journal code: 9426463. ISSN: 1000-3061.

PUB. COUNTRY: China
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Chinese
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 11 Feb 2006
Last Updated on STN: 11 Feb 2006

AB The **phytase** gene phyA(m) from Aspergillus niger N25 was recombined into E. coli expression vector pET-30b(+). Recombined at expression vectors pET30b-FphyA(m) was served as a template to amplify

phytase gene, and the PCR product named elongation mutation gene phyA(e) was expanded with a 13 amino acid sequence from pET-30b-FphyA(m) vector at C-terminal of **phytase** gene phyA(m). Furthermore, phyA(e) gene was recombined into expression vector pPIC9k and expressed in Pichia pastoris. The comparison experiment of **mutant** **phytase** PP-NP0 with wild-type **phytase** PP-NP(m)-8 showed that: the optimum temperature of PP-NPe was increased by 3 degrees C, and its thermostability was increased by 21% when it was exposed to 10 min at 75 degrees C. Its effective reaction pH range with catalysis efficiency above 70% was pH 4.6 - pH 6.6, and wider 0.4 pH value than that of wild-type **phytase**.

L7 ANSWER 4 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2005146188 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15642731
TITLE: The nonconsecutive disulfide bond of Escherichia coli **phytase** (AppA) renders it dependent on the protein-disulfide isomerase, DsbC.
AUTHOR: Berkmen Mehmet; Boyd Dana; Beckwith Jon
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA.
CONTRACT NUMBER: GM-55090 (NIGMS)
SOURCE: The Journal of biological chemistry, (2005 Mar 25) Vol. 280, No. 12, pp. 11387-94. Electronic Publication: 2005-01-10.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200504
ENTRY DATE: Entered STN: 22 Mar 2005
Last Updated on STN: 22 Apr 2005
Entered Medline: 21 Apr 2005

AB The formation of protein disulfide bonds in the Escherichia coli periplasm by the enzyme DsbA is an inaccurate process. Many eukaryotic proteins with nonconsecutive disulfide bonds expressed in E. coli require an additional protein for proper folding, the disulfide bond isomerase DsbC. Here we report studies on a native E. coli periplasmic acid phosphatase, **phytase** (AppA), which contains three consecutive and one nonconsecutive disulfide bonds. We show that AppA requires DsbC for its folding. However, the activity of an AppA **mutant** lacking its nonconsecutive disulfide bond is DsbC-independent. An AppA homolog, Agp, a periplasmic acid phosphatase with similar structure, lacks the nonconsecutive disulfide bond but has the three consecutive disulfide bonds found in AppA. The consecutively disulfide-bonded Agp is not dependent on DsbC but is rendered dependent by engineering into it the conserved nonconsecutive disulfide bond of AppA. Taken together, these results provide support for the proposal that proteins with nonconsecutive disulfide bonds require DsbC for full activity and that disulfide bonds are formed predominantly during translocation across the cytoplasmic membrane.

L7 ANSWER 5 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2004566189 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15537962
TITLE: Phosphorus composition of manure from swine fed low-phytate grains: evidence for hydrolysis in the animal.
AUTHOR: Leytem April B; Turner Benjamin L; Thacker P A
CORPORATE SOURCE: USDA-ARS, Northwest Irrigation and Soils Research Laboratory, 3793 N 3600 E, Kimberly, ID 83341, USA..
leytem@nwisrl.ars.usda.gov
SOURCE: Journal of environmental quality, (2004 Nov-Dec) Vol. 33, No. 6, pp. 2380-3.

Journal code: 0330666. ISSN: 0047-2425.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200502
ENTRY DATE: Entered STN: 13 Nov 2004
Last Updated on STN: 10 Feb 2005
Entered Medline: 9 Feb 2005

AB Including low-phytic-acid grains in swine diets can reduce P concentrations in manure, but the influence on manure P composition is relatively unknown. To address this we analyzed manure from swine fed one of four barley (Hordeum vulgare L.) varieties. The barley types consisted of wild-type barley (CDC bold, normal barley diet) and three low-phytic-acid **mutant** barleys that contained similar amounts of total P but less phytic acid. The phytic acid concentrations in the **mutant** barleys were reduced by 32% (M422), 59% (M635), and 97% (M955) compared with that in the wild-type barley, respectively. Phosphorus concentrations were approximately one-third less in manures from animals fed low-phytic-acid barleys compared with those fed the wild-type variety. Phytic acid constituted up to 55% of the P in feed, but only trace concentrations were detected in NaOH-EDTA extracts of all manures by solution (31)P nuclear magnetic resonance (NMR) spectroscopy. Phosphate was the major P fraction in the manures (86-94% extracted P), with small concentrations of pyrophosphate and simple phosphate monoesters also present. The latter originated mainly from the hydrolysis of phospholipids during extraction and analysis. These results suggest that phytic acid is hydrolyzed in swine, possibly in the hind gut by intestinal microflora before being excreted in feces, even though the animals have little **phytase** activity in the gut and derive little nutritional benefit from phytate P. We conclude that feeding low-phytic-acid grains reduces total manure P concentrations and the manure P is no more soluble than P generated from normal barley diets.

L7 ANSWER 6 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2004229578 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15128565
TITLE: Enhancing the thermal tolerance and gastric performance of a microbial **phytase** for use as a phosphate-mobilizing monogastric-feed supplement.
AUTHOR: Garrett James B; Kretz Keith A; O'Donoghue Eileen; Kerovuo Janne; Kim William; Barton Nelson R; Hazlewood Geoffrey P; Short Jay M; Robertson Dan E; Gray Kevin A
CORPORATE SOURCE: Diversa Corporation, San Diego, California 92121, USA.. jgarrett@diversa.com
SOURCE: Applied and environmental microbiology, (2004 May) Vol. 70, No. 5, pp. 3041-6.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200407
ENTRY DATE: Entered STN: 10 May 2004
Last Updated on STN: 28 Jul 2004
Entered Medline: 27 Jul 2004

AB The inclusion of **phytase** in monogastric animal feed has the benefit of hydrolyzing indigestible plant phytate (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) to provide poultry and swine with dietary phosphorus. An ideal **phytase** supplement should have a high temperature tolerance, allowing it to survive the feed pelleting process, a high specific activity at low pHs, and adequate gastric performance. For this study, the performance of a bacterial **phytase** was optimized by the use of gene site saturation

mutagenesis technology. Beginning with the appA gene from Escherichia coli, a library of clones incorporating all 19 possible amino acid changes and 32 possible codon variations in 431 residues of the sequence was generated and screened for **mutants** exhibiting improved thermal tolerance. Fourteen single site variants were discovered that retained as much as 10 times the residual activity of the wild-type enzyme after a heated incubation regimen. The addition of eight individual mutations into a single construct (Phy9X) resulted in a protein of maximal fitness, i.e., a highly active **phytase** with no loss of activity after heating at 62 degrees C for 1 h and 27% of its initial activity after 10 min at 85 degrees C, which was a significant improvement over the appA parental **phytase**. Phy9X also showed a 3.5-fold enhancement in gastric stability.

L7 ANSWER 7 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2003523790 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14601878
TITLE: Effectiveness of an experimental consensus **phytase** in improving dietary phytate-phosphorus utilization by weanling pigs.
AUTHOR: Gentile J M; Roneker K R; Crowe S E; Pond W G; Lei X G
CORPORATE SOURCE: Department of Animal Science, Cornell University, Ithaca, NY 14853, USA.
SOURCE: Journal of animal science, (2003 Nov) Vol. 81, No. 11, pp. 2751-7.
Journal code: 8003002. ISSN: 0021-8812.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200402
ENTRY DATE: Entered STN: 7 Nov 2003
Last Updated on STN: 13 Feb 2004
Entered Medline: 12 Feb 2004

AB Consensus **phytase** is a new biosynthetic, heat-stable enzyme derived from the sequences of multiple homologous **phytases**. Two experiments were conducted to determine its effectiveness, relative to inorganic P and a **mutant** enzyme of Escherichia coli **phytase** (**Mutant**-EP), in improving dietary phytate-P availability to pigs. In Exp. 1, 36 pigs (3 wk old, 7.00 +/- 0.24 kg of BW) were fed a low-P corn-soybean meal basal diet plus consensus **phytase** at 0, 250, 500, 750, 1,000, or 1,250 U/kg of feed for 5 wk. Plasma inorganic P concentration, plasma alkaline phosphatase activity, bone strength, and overall ADG and gain:feed ratio of pigs were improved ($P < 0.05$) by consensus **phytase** in both linear ($R2 = 0.20$ to 0.70) and quadratic ($R2 = 0.30$ to 0.70) dose-dependent fashions. In Exp. 2, 36 pigs (4 wk old, 9.61 +/- 0.52 kg BW) were fed the basal diet + inorganic P at 0.1 or 0.2%, consensus **phytase** at 750 or 450 U/kg of feed, **Mutant**-EP at 450 U/kg of feed, or 225 U consensus + 225 U **Mutant**-EP/kg of feed. Pigs fed 750 U of consensus **phytase** or 450 U of **Mutant**-EP/kg feed had plasma inorganic concentrations and bone strength that fell between those of pigs fed 0.1 or 0.2% inorganic P. These two measures were 16 to 29% lower ($P < 0.05$) in pigs fed 450 U of consensus **phytase**/kg of feed than those of pigs fed 0.2% inorganic P. Plasma inorganic P concentrations were 14 to 29% higher ($P < 0.05$) in pigs fed **Mutant**-EP vs. consensus **phytase** at 450 U/kg at wk 2 and 3. In conclusion, the experimental consensus **phytase** effectively releases phytate P from the corn-soy diet for weanling pigs. The inorganic P equivalent of 750 U of consensus **phytase**/kg of feed may fall between 0.1 and 0.2%, but this requires further determination.

L7 ANSWER 8 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2003523577 MEDLINE

DOCUMENT NUMBER: PubMed ID: 14601665
TITLE: PhyA, a secreted protein of Xanthomonas oryzae pv. oryzae, is required for optimum virulence and growth on phytic acid as a sole phosphate source.
AUTHOR: Chatterjee Subhadeep; Sankaranarayanan Rajan; Sonti Ramesh V
CORPORATE SOURCE: Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad-500 007, India.
SOURCE: Molecular plant-microbe interactions : MPMI, (2003 Nov) Vol. 16, No. 11, pp. 973-82.
Journal code: 9107902. ISSN: 0894-0282.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AY151260
ENTRY MONTH: 200402
ENTRY DATE: Entered STN: 7 Nov 2003
Last Updated on STN: 11 Feb 2004
Entered Medline: 10 Feb 2004

AB Xanthomonas oryzae pv. oryzae causes bacterial leaf blight, a serious disease of rice. We have identified a novel virulence deficient **mutant** (BXO1691) of X. oryzae pv. oryzae that has a Tn5 insertion in an open reading frame (phyA; putative **phytase** A) encoding a 373-amino acid (aa) protein containing a 28-aa predicted signal peptide. Extracellular protein profiles revealed that a 38-kDa band is absent in phyA **mutants** as compared with phyA+ strains. A BLAST search with phyA and its deduced polypeptide sequence indicated significant similarity with conserved hypothetical proteins in Xanthomonas axonopodis pv. citri and Xanthomonas campestris pv. campestris and limited homology to secreted **phytases** of Bacillus species. Homology modeling with a Bacillus **phytase** as the template suggests that the PhyA protein has a similar six-bladed beta-propeller architecture and exhibits conservation of certain critical active site residues. **Phytases** are enzymes that are involved in degradation of phytic acid (inositol hexaphosphate), a stored form of phosphate in plants. The phyA **mutants** exhibit a growth deficiency in media containing phytic acid as a sole phosphate source. Exogenous phosphate supplementation promotes migration of phyA X. oryzae pv. oryzae **mutants** in rice leaves. These results suggest that the virulence deficiency of phyA **mutants** is, at least in part, due to inability to use host phytic acid as a source of phosphate. phyA-like genes have not been previously reported to be involved in the virulence of any plant pathogenic bacterium.

L7 ANSWER 9 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2003468797 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14523526
TITLE: Phenotypic, genetic and molecular characterization of a maize low phytic acid **mutant** (lpa241).
AUTHOR: Pilu R; Panzeri D; Gavazzi G; Rasmussen S K; Consonni G; Nielsen E
CORPORATE SOURCE: Dipartimento di Produzione Vegetale, Universita degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy.
SOURCE: TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik, (2003 Oct) Vol. 107, No. 6, pp. 980-7.
Electronic Publication: 2003-10-02.
Journal code: 0145600. ISSN: 0040-5752.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200403
ENTRY DATE: Entered STN: 8 Oct 2003

Last Updated on STN: 13 Mar 2004
Entered Medline: 12 Mar 2004

AB Phytic acid, myo-inositol 1,2,3,4,5,6-hexakisphosphate, is the major storage compound of phosphorous (P) in plants, predominantly accumulating in seeds (up to 4-5% of dry weight) and pollen. In cereals, phytic acid is deposited in embryo and aleurone grain tissues as a mixed "phytate" salt of potassium and magnesium, although phytates contain other mineral cations such as iron and zinc. During germination, phytates are broken down by the action of **phytases**, releasing their P, minerals and myo-inositol which become available to the growing seedling. Phytic acid represents an anti-nutritional factor for animals, and isolation of maize low phytic acid (lpa) **mutants** provides a novel approach to study its biochemical pathway and to tackle the nutritional problems associated with it. Following chemical mutagenesis of pollen, we have isolated a viable recessive **mutant** named lpa 241 showing about 90% reduction of phytic acid and about a tenfold increase in seed-free phosphate content. Although germination rate was decreased by about 30% compared to wild-type, developement of **mutant** plants was apparentely unaffected. The results of the genetic, biochemical and molecular characterization experiments carried out by SSR mapping, MDD-HPLC and RT-PCR are consistent with a mutation affecting the MIPS1S gene, coding for the first enzyme of the phytic acid biosynthetic pathway.

L7 ANSWER 10 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2002465993 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12226725
TITLE: Overexpression of the **phytase** from Escherichia coli and its extracellular production in bioreactors.
AUTHOR: Miksch G; Kleist S; Friehs K; Flaschel E
CORPORATE SOURCE: Department of Fermentation Engineering, Faculty of Technology, University of Bielefeld, 33501 Bielefeld, Germany.. gmi@fermtech.techfak.uni-bielefeld.de
SOURCE: Applied microbiology and biotechnology, (2002 Sep) Vol. 59, No. 6, pp. 685-94. Electronic Publication: 2002-07-17. Journal code: 8406612. ISSN: 0175-7598.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200212
ENTRY DATE: Entered STN: 13 Sep 2002
Last Updated on STN: 5 Jan 2003
Entered Medline: 10 Dec 2002

AB The gene for **phytase** from Escherichia coli was sequenced and compared with the appA gene. It was found to be a **mutant** derivative of the appA gene. After fusion with a C-terminal His-tag, **phytase** was purified by affinity chromatography and the enzymatic properties were analyzed. To develop a system for overexpression and extracellular production of **phytase** in E. coli, factors affecting the expression and secretion such as promoter type, host strain and selection pressure were analyzed. Using a secretion system based on the controlled expression of the kil gene, the expression of **phytase** was improved and the enzyme was released into the culture medium at a high level. An effective fermentation strategy based on fed-batch operation was developed.

L7 ANSWER 11 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2002424721 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12180922
TITLE: Proteomic analysis of the Caulobacter crescentus stalk indicates competence for nutrient uptake.
AUTHOR: Ireland Marcia M E; Karty Jonathan A; Quardokus Ellen M; Reilly James P; Brun Yves V
CORPORATE SOURCE: Department of Biology, Indiana University, Bloomington, IN

47405, USA.
CONTRACT NUMBER: GM51986 (NIGMS)
GM61336 (NIGMS)
SOURCE: Molecular microbiology, (2002 Aug) Vol. 45, No. 4, pp. 1029-41.
Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200210
ENTRY DATE: Entered STN: 16 Aug 2002
Last Updated on STN: 31 Oct 2002
Entered Medline: 30 Oct 2002

AB Caulobacter crescentus, a Gram-negative alpha-purple proteobacterium, is an oligotroph that lives in aquatic environments dilute in nutrients. This bacterium divides asymmetrically. Part of this asymmetric cell division involves the formation of a prosthecum at one pole, referred to as the stalk, which replaces the flagellum of the motile swarmer cell. Little is known about the synthesis or function of the stalk. The stalk is an extension of the cell membranes and peptidoglycan layer, and stalk elongation is stimulated by phosphate starvation. In this study, we have taken advantage of two-dimensional gel (2D gel) electro-phoresis as well as the fully sequenced genome of Caulobacter to study the proteome of the stalk. We modified a stalk-shedding **mutant** strain of Caulobacter crescentus to increase the yield of stalk material shed and performed 2D gel electrophoresis of purified stalks and cellular fractions. Comparison of the stalk 2D gel with the 2D gels of cell membrane and soluble fractions showed that the stalk is mostly free of cytoplasmic proteins and has a profile very similar to that of the cell membrane. Of the 172 proteins on a stalk 2D gel, we report the identification of 64 spots, corresponding to 39 different proteins present in the stalk of Caulobacter. The identifications include several TonB-dependent receptors, two OmpA family proteins, a dipeptidase, GlpQ, two alkaline phosphatases, 3-**phytase**, a putative TolC protein and 11 proteins of unknown function. These identifications are consistent with the hypothesis that the stalk plays a role in nutrient uptake.

L7 ANSWER 12 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2002411702 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12101298
TITLE: Extracellular **phytase** activity of Bacillus amyloliquefaciens FZB45 contributes to its plant-growth-promoting effect.
AUTHOR: Idriss Elsorra E; Makarewicz Oliwia; Farouk Abdelazim; Rosner Kristin; Greiner Ralf; Bochow Helmut; Richter Thomas; Borriss Rainer
CORPORATE SOURCE: Humboldt Universitat Berlin, Institut fur Biologie, Chaussee-Strasse 117, D-10115 Berlin, Germany.
SOURCE: Microbiology (Reading, England), (2002 Jul) Vol. 148, No. Pt 7, pp. 2097-109.
Journal code: 9430468. ISSN: 1350-0872.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AY055219; GENBANK-AY055220; GENBANK-AY055221; GENBANK-AY055222; GENBANK-AY055223; GENBANK-AY055224; GENBANK-AY055225; GENBANK-AY055226
ENTRY MONTH: 200210
ENTRY DATE: Entered STN: 9 Aug 2002
Last Updated on STN: 22 Oct 2002
Entered Medline: 21 Oct 2002

AB Several Bacillus strains belonging to the B. subtilis/amyloliquefaciens

group isolated from plant-pathogen-infested soil possess plant-growth-promoting activity [Krebs, B. et al. (1998) J Plant Dis Prot 105, 181-197]. Three out of the four strains investigated were identified as B. amyloliquefaciens and were able to degrade extracellular phytate (myo-inositol hexakisphosphate). The highest extracellular **phytase** activity was detected in strain FZB45, and diluted culture filtrates of this strain stimulated growth of maize seedlings under phosphate limitation in the presence of phytate. The amino acid sequence deduced from the **phytase** phyA gene cloned from FZB45 displayed a high degree of similarity to known Bacillus **phytases**. Weak similarity between FZB45 **phytase** and B. subtilis alkaline phosphatase IV pointed to a possible common origin of these two enzymes. The recombinant protein expressed by B. subtilis MU331 displayed 3(1)-**phytase** activity yielding D/L-Ins(1,2,4,5,6)P5 as the first product of phytate hydrolysis. A **phytase**-negative **mutant** strain, FZB45/M2, whose phyA gene is disrupted, was generated by replacing the entire wild-type gene on the chromosome of FZB45 with a km::phyA fragment, and culture filtrates obtained from FZB45/M2 did not stimulate plant growth. In addition, the growth of maize seedlings was promoted in the presence of purified **phytase** and the absence of culture filtrate. These genetic and biochemical experiments provide strong evidence that **phytase** activity of B. amyloliquefaciens FZB45 is important for plant growth stimulation under phosphate limitation.

L7 ANSWER 13 OF 25 MEDLINE on STN

ACCESSION NUMBER: 2002346685 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12034860
TITLE: The consensus concept for thermostability engineering of proteins: further proof of concept.
AUTHOR: Lehmann Martin; Loch Claudia; Middendorf Anke; Studer Dominik; Lassen Soren F; Pasamontes Luis; van Loon Adolphus P G M; Wyss Markus
CORPORATE SOURCE: Roche Vitamins AG, Department VFB, Building 203/112a, CH-4070 Basel, Switzerland.. martin.lehmann@roche.com
SOURCE: Protein engineering, (2002 May) Vol. 15, No. 5, pp. 403-11. Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200307
ENTRY DATE: Entered STN: 2 Jul 2002
Last Updated on STN: 11 Dec 2002
Entered Medline: 11 Jul 2003

AB Previously, we calculated a consensus amino acid sequence from 13 homologous fungal **phytases**. A synthetic gene was constructed and recombinantly expressed. Surprisingly, consensus **phytase**-1 was 15-26 degrees C more thermostable than all parent **phytases** used in its design [Lehmann et al. (2000)Protein English, 13, 49-57]. In the present study, inclusion of six further **phytase** sequences in the amino acid sequence alignment resulted in the replacement of 38 amino acid residues in either one or both of the new consensus **phytases**-10 and -11. Since consensus **phytase**-10, again, was 7.4 degrees C more thermostable than consensus **phytase**-1, the thermostability effects of most of the 38 amino acid substitutions were tested by site-directed mutagenesis. Both stabilizing and destabilizing mutations were identified, but all affected the stability of the enzyme by <3 degrees C. The combination of all stabilizing amino acid exchanges in a multiple **mutant** of consensus **phytase**-1 increased the unfolding temperature from 78.0 to 88.5 degrees C. Likewise, back-mutation of four destabilizing amino acids and introduction of an additional stabilizing amino acid in consensus **phytase**-10 further increased the unfolding temperature from 85.4 to 90.4 degrees C.

The thermostabilization achieved is the result of a combination of slight improvements from multiple amino acid exchanges rather than being the effect of a single or of just a few dominating mutations that have been introduced by chance. The present findings support the general validity of the consensus concept for thermostability engineering of proteins.

L7 ANSWER 14 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2002215248 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11916711
TITLE: Engineering of **phytase** for improved activity at low pH.
AUTHOR: Tomschy Andrea; Brugger Roland; Lehmann Martin; Svendsen Allan; Vogel Kurt; Kostrewa Dirk; Lassen Soren F; Burger Dominique; Kronenberger Alexandra; van Loon Adolphus P G M; Pasamontes Luis; Wyss Markus
CORPORATE SOURCE: Biotechnology Department, Roche Vitamins, Ltd., 4070 Basel, Switzerland.
SOURCE: Applied and environmental microbiology, (2002 Apr) Vol. 68, No. 4, pp. 1907-13.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 16 Apr 2002
Last Updated on STN: 13 Jul 2002
Entered Medline: 12 Jul 2002

AB For industrial applications in animal feed, a **phytase** of interest must be optimally active in the pH range prevalent in the digestive tract. Therefore, the present investigation describes approaches to rationally engineer the pH activity profiles of Aspergillus fumigatus and consensus **phytases**. Decreasing the negative surface charge of the A. fumigatus Q27L **phytase mutant** by glycinamidylation of the surface carboxy groups (of Asp and Glu residues) lowered the pH optimum by ca. 0.5 unit but also resulted in 70 to 75% inactivation of the enzyme. Alternatively, detailed inspection of amino acid sequence alignments and of experimentally determined or homology modeled three-dimensional structures led to the identification of active-site amino acids that were considered to correlate with the activity maxima at low pH of A. niger NRRL 3135 **phytase**, A. niger pH 2.5 acid phosphatase, and Peniophora lycii **phytase**. Site-directed mutagenesis confirmed that, in A. fumigatus wild-type **phytase**, replacement of Gly-277 and Tyr-282 with the corresponding residues of A. niger **phytase** (Lys and His, respectively) gives rise to a second pH optimum at 2.8 to 3.4. In addition, the K68A single mutation (in both A. fumigatus and consensus **phytase** backbones), as well as the S140Y D141G double mutation (in A. fumigatus **phytase** backbones), decreased the pH optima with phytic acid as substrate by 0.5 to 1.0 unit, with either no change or even a slight increase in maximum specific activity. These findings significantly extend our tools for rationally designing an optimal **phytase** for a given purpose.

L7 ANSWER 15 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2002156399 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11888166
TITLE: Regulation of Raoultella terrigena comb.nov. **phytase** expression.
AUTHOR: Zamudio Marcela; Gonzalez Aracely; Bastarrachea Fernando
CORPORATE SOURCE: Facultad de Ingenieria Quimica, Universidad Autonoma de Yucatan, Merida, Mexico.. zmaya@tunku.uady.mx
SOURCE: Canadian journal of microbiology, (2002 Jan) Vol. 48, No. 1, pp. 71-81.

Journal code: 0372707. ISSN: 0008-4166.
PUB. COUNTRY: Canada
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF427147
ENTRY MONTH: 200209
ENTRY DATE: Entered STN: 13 Mar 2002
Last Updated on STN: 26 Sep 2002
Entered Medline: 25 Sep 2002

AB **Phytases** catalyze the release of phosphate from phytate (myo-inositol hexakisphosphate) to inositol polyphosphates. Raoultella terrigena comb.nov. **phytase** activity is known to increase markedly after cells reach the stationary phase. In this study, **phytase** activity measurements made on single batch cultures indicated that specific enzyme activity was subject to catabolite repression. Cyclic AMP (cAMP) showed a positive effect in expression during exponential growth and a negative effect during stationary phase. RpoS exhibited the opposite effect during both growth phases; the induction to stationary phase decreased twofold in the rpoS::Tn10 **mutant**, but the effect of RpoS was not clearly determined. Two phy::MudI1734 **mutants**, MW49 and MW52, were isolated. These formed small colonies in comparison with the MW25 parent strain when plated on Luria-Bertani (LB) or LB supplemented with glucose. They did not grow in minimal media or under anaerobiosis, but did grow aerobically on LB and LB glucose at a lower rate than did MW25. The beta-galactosidase activity level in these **mutants** increased three to four fold during stationary growth in LB glucose and during anaerobiosis. Addition of cAMP during the exponential growth of MW52 on LB glucose provoked a decrease in beta-galactosidase activity during the stationary phase, confirming its negative effect on **phytase** expression during stationary growth.

L7 ANSWER 16 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2001568769 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11312780
TITLE: Nutritionally relevant parameters in low-phytate barley (hordeumvulgare L.) grain **mutants**.
AUTHOR: Hatzack F; Johansen K S; Rasmussen S K
CORPORATE SOURCE: Plant Products and Biomass Recycling Program, Plant Biology and Biogeochemistry Department, PBK-301, Riso National Laboratory, Post Office Box 49, DK-4000 Roskilde, Denmark.
SOURCE: Journal of agricultural and food chemistry, (2000 Dec) Vol. 48, No. 12, pp. 6074-80.
Journal code: 0374755. ISSN: 0021-8561.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 29 Oct 2001
Last Updated on STN: 29 Oct 2001
Entered Medline: 25 Oct 2001

AB Nutritionally relevant parameters in barley low-phytate **mutant** grains were analyzed in order to assess the potential value of these lines for future feeding trials. Phytate (myo-inositol 1,2,3,4,5,6-hexakisphosphate) levels in grains from A- and B-type low-phytate **mutants** corresponded to 25% and 66% of those of the parent line content, respectively. These relative decreases in phytate were accompanied by proportional increases of inorganic phosphate amounts. Apart from phytate, A-type grains also contained substantial quantities of myo-inositol 1,3,4,5-tetrakisphosphate. Phytate levels in straw and root material from **mutants** were similar to parent line controls, indicating that low-phytate mutations were grain specific. Analysis of K,

Mg, Ca, and Zn revealed normal or slightly increased mineral cation levels in grains from all low-phytate lines, suggesting that mutationally impaired phytate accumulation did not affect mineral storage capacity. Other nutritionally important parameters such as starch and protein contents were similar to parent line controls. Finally, dynamic changes in the phosphorus composition during kernel development suggested that A-type mutations directly affected phytate synthesis, whereas B-type mutations seemed to act on regulation of synthesis.

L7 ANSWER 17 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2001195126 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11171128
TITLE: Inositol phosphates from barley low-phytate grain **mutants** analysed by metal-dye detection HPLC and NMR.
AUTHOR: Hatzack F; Hubel F; Zhang W; Hansen P E; Rasmussen S K
CORPORATE SOURCE: Plant Products and Biomass Recycling Programme, Plant Biology and Biogeochemistry Department, PBK-301, Riso National Laboratory, P.O. Box 49, DK-4000 Roskilde, Denmark.
SOURCE: The Biochemical journal, (2001 Mar 1) Vol. 354, No. Pt 2, pp. 473-80.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 10 Apr 2001
Last Updated on STN: 10 Apr 2001
Entered Medline: 5 Apr 2001

AB Inositol phosphates from barley low-phytate grain **mutants** and their parent variety were analysed by metal-dye detection HPLC and NMR. Compound assignment was carried out by comparison of retention times using a chemical hydrolysate of phytate [Ins(1,2,3,4,5,6)P(6)] as a reference. Co-inciding retention times indicated the presence of phytate, D/L-Ins(1,2,3,4,5)P(5), Ins(1,2,3,4,6)P(5), D/L-(1,2,4,5,6)P(5), D/L-(1,2,3,4)P(4), D/L-Ins(1,2,5,6)P(4) and D/L-Ins(1,4,5,6)P(4) in PLP1B **mutants** as well as the parent variety. In grain extracts from **mutant** lines PLP1A, PLP2A and PLP3A unusual accumulations of D/L-Ins(1,3,4,5)P(4) were observed whereas phytate and the above-mentioned inositol phosphates were present in relatively small amounts. Assignment of D/L-Ins(1,3,4,5)P(4) was corroborated by precise co-chromatography with a commercial Ins(1,3,4,5)P(4) standard and by NMR spectroscopy. Analysis of inositol phosphates during grain development revealed accumulation of phytate and D/L-Ins(1,3,4,5)P(4), which suggested the tetrakisphosphate compound to be an intermediate of phytate synthesis. This assumption was strengthened further by phytate degradation assays showing that D/L-Ins(1,3,4,5)P(4) did not belong to the spectrum of degradation products generated by endogenous **phytase** activity. Metabolic scenarios leading to accumulation of D/L-Ins(1,3,4,5)P(4) in barley low-phytate **mutants** are discussed.

L7 ANSWER 18 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2001150278 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11164958
TITLE: Structure-based chimeric enzymes as an alternative to directed enzyme evolution: **phytase** as a test case.
AUTHOR: Jermutus L; Tessier M; Pasamontes L; van Loon A P; Lehmann M
CORPORATE SOURCE: F. Hoffmann-La Roche, Vitamins and Fine Chemicals Division, 4070, Basel, Switzerland.
SOURCE: Journal of biotechnology, (2001 Jan 23) Vol. 85, No. 1, pp.

15-24.
Journal code: 8411927. ISSN: 0168-1656.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 4 Apr 2001
Last Updated on STN: 4 Apr 2001
Entered Medline: 15 Mar 2001

AB Thermostability is a key feature for commercially attractive variants of the fungal enzyme **phytase**. In an initial set of experiments, we restored ionic interactions and hydrogen bonds on the surface of Aspergillus terreus **phytase**, which are present in the homologous but more thermostable enzyme from A. niger. Since these mutations turned out to be neutral, we replaced-in the same region and based on the crystal structure of A. niger **phytase**-entire secondary structure elements. The replacement of one alpha-helix on the surface of A. terreus **phytase** by the corresponding stretch of A. niger **phytase** resulted in an enzyme with improved thermostability and unaltered enzymatic activity. Surprisingly, the thermostability of this hybrid protein was very similar to that of A. niger **phytase**, although the fusion protein contained only a 31 amino acid stretch of the more stable parent enzyme. This report provides evidence that structure-based chimeric enzymes can be used to exploit the evolutionary information within a sequence alignment. We propose this method as an alternative to directed enzyme evolution if due to expression constraints the screening of large **mutant** populations is not feasible.

L7 ANSWER 19 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2000502660 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11051103
TITLE: Site-directed mutagenesis improves catalytic efficiency and thermostability of Escherichia coli pH 2.5 acid phosphatase/**phytase** expressed in Pichia pastoris.
AUTHOR: Rodriguez E; Wood Z A; Karplus P A; Lei X G
CORPORATE SOURCE: Department of Animal Science, Cornell University, Ithaca, New York 14853, USA.
SOURCE: Archives of biochemistry and biophysics, (2000 Oct 1) Vol. 382, No. 1, pp. 105-12.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 22 Mar 2001
Last Updated on STN: 22 Mar 2001
Entered Medline: 13 Nov 2000

AB Escherichia coli pH 2.5 acid phosphatase gene (appA) and three **mutants** were expressed in Pichia pastoris to assess the effect of strategic mutations or deletion on the enzyme (EcAP) biochemical properties. **Mutants** A131N/ V134N/D207N/S211N, C200N/D207N/S211N, and A131N/ V134N/C200N/D207N/S211N had four, two, and four additional potential N-glycosylation sites, respectively. Extracellular **phytase** and acid phosphatase activities were produced by these **mutants** and the intact enzyme r-AppA. The N-glycosylation level was higher in **mutants** A131N/V134N/D207N/S211N (48%) and A131N/V134N/ C200N/D207N/S211N (89%) than that in r-AppA (14%). Despite no enhancement of glycosylation, **mutant** C200N/ D207N/S211N was different from r-AppA in the following properties. First, it was more active at pH 3.5-5.5. Second, it retained more ($P < 0.01$) **phytase** activity than that of r-AppA. Third, its specific activity of **phytase** was 54% higher.

Lastly, its apparent catalytic efficiency kcat/Km for either p-nitrophenyl phosphate (5.8 x 10(5) vs 2.0 x 10(5) min(-1) M(-1)) or sodium phytate (6.9 x 10(6) vs 1.1 x 10(6) min(-1) M(-1)) was improved by factors of 1.9- and 5.3-fold, respectively. Based on the recently published E. coli **phytase** crystal structure, substitution of C200N in **mutant** C200N/D207N/S211N seems to eliminate the disulfide bond between the G helix and the GH loop in the alpha-domain of the protein. This change may modulate the domain flexibility and thereby the catalytic efficiency and thermostability of the enzyme.

L7 ANSWER 20 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2000161462 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10696472
TITLE: Characterization and overproduction of the Escherichia coli appA encoded bifunctional enzyme that exhibits both **phytase** and acid phosphatase activities.
AUTHOR: Golovan S; Wang G; Zhang J; Forsberg C W
CORPORATE SOURCE: Department of Microbiology, University of Guelph, Canada.
SOURCE: Canadian journal of microbiology, (2000 Jan) Vol. 46, No. 1, pp. 59-71.
Journal code: 0372707. ISSN: 0008-4166.
PUB. COUNTRY: Canada
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 5 May 2000
Last Updated on STN: 5 May 2000
Entered Medline: 25 Apr 2000

AB The appA gene that was previously shown to code for an acid phosphatase instead codes for a bifunctional enzyme exhibiting both acid phosphatase and **phytase** activities. The purified enzyme with a molecular mass of 44,708 Da was further separated by chromatofocusing into two isoforms of identical size with isoelectric points of 6.5 and 6.3. The isoforms had identical pH optima of 4.5 and were stable at pH values from 2 to 10. The temperature optimum for both **phytase** isoforms was 60 degrees C. When heated at different pH values the enzyme showed the greatest thermal resistance at pH 3. The pH 6.5 isoform exhibited K(m) and Vmax values of 0.79 mM and 3165 U.mg-1 of protein for **phytase** activity and 5.5 mM and 712 U.mg-1 of protein for acid phosphatase, respectively. The pH 6.3 isoform exhibited slightly lower K(m) and Vmax values. The enzyme exhibited similar properties to the **phytase** purified by Greiner et al. (1993), except the specific activity of the enzyme was at least 3.5-fold less than that previously reported, and the N-terminal amino acid sequence was different. The Bradford assay, which was used by Greiner et al. (1993) for determination of enzyme concentration was, in our hands, underestimating protein concentration by a factor of 14. **Phytase** production using the T7 polymerase expression system was enhanced by selection of a **mutant** able to grow in a chemically defined medium with lactose as the carbon source and inducer. Using this strain in fed-batch fermentation, **phytase** production was increased to over 600 U.mL-1. The properties of the **phytase** including the low pH optimum, protease resistance, and high activity, demonstrates that the enzyme is a good candidate for industrial production as a feed enzyme.

L7 ANSWER 21 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2000140172 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10677002
TITLE: High-performance thin-layer chromatography method for inositol phosphate analysis.
AUTHOR: Hatzack F; Rasmussen S K
CORPORATE SOURCE: Plant Biology and Biogeochemistry Department, Riso National Laboratory, Roskilde, Denmark.. frank.hatzack@risoe.dk

SOURCE: Journal of chromatography. B, Biomedical sciences and applications, (1999 Dec 24) Vol. 736, No. 1-2, pp. 221-9.
Journal code: 9714109. ISSN: 1387-2273.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20 Mar 2000
Last Updated on STN: 20 Mar 2000
Entered Medline: 7 Mar 2000

AB A simple and inexpensive high-performance thin-layer chromatography (HPTLC) method for the analysis of inositol mono- to hexakisphosphates on cellulose precoated plates is described. Plates were developed in 1-propanol-25% ammonia solution-water (5:4:1) and substance quantities as low as 100-200 pmol were detected by molybdate staining. Chromatographic mobilities of nucleotides and phosphorylated carbohydrates were also characterized. Charcoal treatment was employed to separate nucleotides from inositol phosphates with similar R(F) values prior to HPTLC analysis. Practical application of the HPTLC system is demonstrated by analysis of grain extracts from wild type and low-phytate **mutant** barley as well as phytate degradation products resulting from barley **phytase** activity.

L7 ANSWER 22 OF 25 MEDLINE on STN
ACCESSION NUMBER: 1999124560 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9925554
TITLE: Biophysical characterization of fungal **phytases** (myo-inositol hexakisphosphate phosphohydrolases): molecular size, glycosylation pattern, and engineering of proteolytic resistance.
AUTHOR: Wyss M; Pasamontes L; Friedlein A; Remy R; Tessier M; Kronenberger A; Middendorf A; Lehmann M; Schnoebelen L; Rothlisberger U; Kusznir E; Wahl G; Muller F; Lahm H W; Vogel K; van Loon A P
CORPORATE SOURCE: VFB Department, F. Hoffmann-La Roche Ltd., CH-4070 Basel, Switzerland.. markus.wyss@roche.com
SOURCE: Applied and environmental microbiology, (1999 Feb) Vol. 65, No. 2, pp. 359-66.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 2 Apr 1999
Last Updated on STN: 2 Apr 1999
Entered Medline: 24 Mar 1999

AB **Phytases** (myo-inositol hexakisphosphate phosphohydrolases) are found naturally in plants and microorganisms, particularly fungi. Interest in these enzymes has been stimulated by the fact that **phytase** supplements increase the availability of phosphorus in pig and poultry feed and thereby reduce environmental pollution due to excess phosphate excretion in areas where there is intensive livestock production. The wild-type **phytases** from six different fungi, Aspergillus niger, Aspergillus terreus, Aspergillus fumigatus, Emericella nidulans, Myceliophthora thermophila, and Talaromyces thermophilus, were overexpressed in either filamentous fungi or yeasts and purified, and their biophysical properties were compared with those of a **phytase** from Escherichia coli. All of the **phytases** examined are monomeric proteins. While E. coli **phytase** is a nonglycosylated enzyme, the glycosylation patterns of the fungal **phytases** proved to be highly variable, differing for individual **phytases**, for a given **phytase** produced in different expression systems, and for

individual batches of a given **phytase** produced in a particular expression system. Whereas the extents of glycosylation were moderate when the fungal **phytases** were expressed in filamentous fungi, they were excessive when the **phytases** were expressed in yeasts. However, the different extents of glycosylation had no effect on the specific activity, the thermostability, or the refolding properties of individual **phytases**. When expressed in A. niger, several fungal **phytases** were susceptible to limited proteolysis by proteases present in the culture supernatant. N-terminal sequencing of the fragments revealed that cleavage invariably occurred at exposed loops on the surface of the molecule. Site-directed mutagenesis of A. fumigatus and E. nidulans **phytases** at the cleavage sites yielded **mutants** that were considerably more resistant to proteolytic attack. Therefore, engineering of exposed surface loops may be a strategy for improving **phytase** stability during feed processing and in the digestive tract.

L7 ANSWER 23 OF 25 MEDLINE on STN
ACCESSION NUMBER: 96128079 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8554538
TITLE: Nucleus-associated phosphorylation of Ins(1,4,5)P3 to InsP6 in Dictyostelium.
AUTHOR: Van der Kaay J; Wesseling J; Van Haastert P J
CORPORATE SOURCE: Department of Biochemistry, University of Groningen, The Netherlands.
SOURCE: The Biochemical journal, (1995 Dec 15) Vol. 312 (Pt 3), pp. 911-7.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199602
ENTRY DATE: Entered STN: 6 Mar 1996
Last Updated on STN: 3 Feb 1997
Entered Medline: 20 Feb 1996

AB Although many cells contain large amounts of InsP6, its metabolism and function is still largely unknown. In Dictyostelium lysates, the formation of InsP6 by sequential phosphorylation of inositol via Ins(3,4,6)P3 has been described [Stevens and Irvine (1990) Nature (London) 346, 580-583]; the second messenger Ins(1,4,5)P3 was excluded as a potential substrate or intermediate for InsP6 formation. However, we observed that **mutant** cells labelled in vivo with [3H]inositol showed altered labelling of both [3H]Ins(1,4,5)P3 and [3H]InsP6. In this report we demonstrate that Ins(1,4,5)P3 is converted into InsP6 in vitro by nucleus-associated enzymes, in addition to the previously described stepwise phosphorylation of inositol to InsP6 that occurs in the cytosol. HPLC analysis indicates that Ins(1,4,5)P3 is converted into InsP6 via sequential phosphorylation at the 3-, 6- and 2-positions. Ins[32P]P6, isolated from cells briefly labelled with [32P]Pi, was analysed using Paramecium **phytase**, which removes the phosphates of InsP6 in a specific sequence. The 6-position contained significantly more 32P radioactivity than the 4- or 5-positions, indicating that the 6-position is phosphorylated after the other two positions. The results from these in vivo and in vitro experiments demonstrate a metabolic route involving the phosphorylation of Ins(1,4,5)P3 via Ins(1,3,4,5)P4 and Ins(1,3,4,5,6)P5 to InsP6 in a nucleus-associated fraction of Dictyostelium cells.

L7 ANSWER 24 OF 25 MEDLINE on STN
ACCESSION NUMBER: 96102019 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8530362
TITLE: A novel, phospholipase C-independent pathway of inositol 1,4,5-trisphosphate formation in Dictyostelium and rat

liver.
AUTHOR: Van Dijken P; de Haas J R; Craxton A; Erneux C; Shears S B; Van Haastert P J
CORPORATE SOURCE: Department of Biochemistry, University of Groningen, The Netherlands.
SOURCE: The Journal of biological chemistry, (1995 Dec 15) Vol. 270, No. 50, pp. 29724-31.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199601
ENTRY DATE: Entered STN: 20 Feb 1996
Last Updated on STN: 3 Feb 1997
Entered Medline: 26 Jan 1996

AB In an earlier study a **mutant** Dictyostelium cell-line (plc-) was constructed in which all phospholipase C activity was disrupted and nonfunctional, yet these cells had nearly normal Ins(1,4,5)P3 levels (Drayer, A.L., Van Der Kaay, J., Mayr, G.W, Van Haastert, P.J.M. (1990) EMBO J. 13, 1601-1609). We have now investigated if these cells have a phospholipase C-independent de novo pathway of Ins(1,4,5)P3 synthesis. We found that homogenates of plc- cells produce Ins(1,4,5)P3 from endogenous precursors. The enzyme activities that performed these reactions were located in the particulate cell fraction, whereas the endogenous substrate was soluble and could be degraded by **phytase**. We tested various potential inositol polyphosphate precursors and found that the most efficient were Ins(1,3,4,5,6)P5, Ins(1,3,4,5)P4, and Ins(1,4,5,6)P4. The utilization of Ins(1,3,4,5,6)P5, which can be formed independently of phospholipase C by direct phosphorylation of inositol (Stephens, L.R. and Irvine, R.F. (1990) Nature 346, 580-582), provides Dictyostelium with an alternative and novel pathway of de novo Ins(1,4,5)P3 synthesis. We further discovered that Ins(1,3,4,5,6)P5 was converted to Ins(1,4,5)P3 via both Ins(1,3,4,5)P4 and Ins(1,4,5,6)P4. In the absence of calcium no Ins(1,4,5)P3 formation could be observed; half-maximal activity was observed at low micromolar calcium concentrations. These reaction steps could also be performed by a single enzyme purified from rat liver, namely, the multiple inositol polyphosphate phosphatase. These data indicate that organisms as diverse as rat and Dictyostelium possess enzyme activities capable of synthesizing the second messengers Ins(1,4,5)P3 and Ins(1,3,4,5)P4 via a novel phospholipase C-independent pathway.

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AB Four acid phosphatase (phosphomonoesterase E.C.3.1.3.2) genes were cloned by polymerase chain reaction (PCR). These were pho3, pho5 and pho11 from

Saccharomyces cerevisiae and the gene for a phosphate-respressible acid phosphatase from Aspergillus niger. The individual genes were subcloned into an A. oryzae expression vector downstream from a starch-inducible alpha-amylase promoter and the resulting expression constructs were transformed into a **mutant** strain of A. oryzae, AO7. Southern hybridization analysis confirmed that the acid phosphatase genes had been integrated into the host genome with estimates of integrated copy numbers ranging from 2 to 20 for individual transformants. Northern hybridization analysis of total RNA from individual transformants revealed the presence of a single transcript of the expected size of 1.8 kb. Production of recombinant protein was induced by the addition of 30 g L-1 of soluble starch in the fermentation media. Active acid phosphatases, not present in control cultures, were detected in the supernatant fractions of transformant cultures by acid phosphatase activity staining of non-denaturing polyacrylamide gels. The ability of the recombinant acid phosphatases to hydrolyze phytate was assessed by referenced **phytase** (myoinositol hexakisphosphate phosphohydrolase E.C. 3.1.3.8) activity assay procedures. A two- to six-fold increase in **phytase** activity was measured in transformants compared to control, untransformed A. oryzae. Sufficient quantities of A. niger and pho5 recombinant acid phosphatases were generated from large-scale fermentations to assess the efficacy of these enzymes as phytate-degrading enzymes when included in poultry diets.(ABSTRACT TRUNCATED AT 250 WORDS)